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I, DAVID DANIEL CLARKE, ASSISTANT DIRECTOR PATENT SERVICES, hereby certify that the annexed are true copies of the Provisional specification and drawing(s) as filed on 30 November 1995 in connection with Application No. PN 6911 for a patent by DARATECH PTY. LTD. filed on 30 November 1995.

I further certify that the name of the applicant has been amended to DARATECH PTY. LTD., and PIG RESEARCH AND DEVELOPMENT CORPORATION pursuant to the provisions of Section 104 of the Patents Act 1990.

I further certify that the annexed documents are not, as yet, open to public inspection.

PRIORITY DOCUMENT



WITNESS my hand this Eighteenth  
day of December 1996

A handwritten signature in cursive script, reading 'D Clarke'.

DAVID DANIEL CLARKE  
ASSISTANT DIRECTOR PATENT SERVICES

AUSTRALIAN	
PROVISIONAL No.	DATE OF FILING
PN6911	30 NOV. 95
PATENT OFFICE	

DARATECH PTY. LTD.

AND  
Pig Research and Development  
Corporation

A U S T R A L I A  
Patents Act 1990

PROVISIONAL SPECIFICATION  
for the invention entitled:



"THERAPEUTIC AND DIAGNOSTIC COMPOSITIONS - B"

The invention is described in the following statement:

## THERAPEUTIC AND DIAGNOSTIC COMPOSITIONS - B

5 The present invention relates generally to therapeutic compositions for the treatment and/or prophylaxis of intestinal disease conditions in animals and birds caused or exacerbated by *Lawsonia intracellularis* or similar organism. The present invention also contemplates methods for the treatment and/or prophylaxis of such intestinal diseases and to diagnostic agents and procedures for detecting *Lawsonia intracellularis* or similar organism.

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Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

15

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

20

The meat industry in Australia and, indeed, in most countries of the world, is an important aspect of the overall livestock industry. However, the meat industry is subject to rapid economic downturn in response to disease conditions affecting the animals as well as human diseases putatively carried by the animals. It is important, therefore, to have well defined treatment, prophylactic and diagnostic procedures available to deal with infections or potential infections in animals and humans.

30 Pigs form a major component of the meat industry. However, pigs are sensitive to a wide spectrum of colonic diseases collectively referred to as porcine proliferative enteritis (PPE). This disease has previously been known as intestinal adenomatosis complex (1), porcine intestinal adenomatosis, necrotic enteritis (2), proliferative haemorrhagic enteropathy (3), regional ileitis (4), haemorrhagic bowel syndrome (5),

porcine proliferative enteropathies and *Campylobacter* spp induced enteritis (6). There are two main forms of PPE: a non-haemorrhagic form represented by intestinal adenomatosis which frequently causes growth retardation and mild diarrhoea; and a haemorrhagic form, which is often fatal, represented by proliferative haemorrhagic enteropathy (PHE) where the distal small intestine lumen becomes engorged with blood. PPE has been reported in a number of animal species including pigs (14), hamsters (7), ferrets (15), guinea pigs (16), rabbits (17) as well as avian species (18).

The causative organism of PPE is a *Campylobacter*-like organism referred to herein as "*Lawsonia intracellularis*" (26). The organism is also previously referred to as *Ileal symbiont intracellularis* (7). PPE-like diseases in pigs may also be caused by other pathogens such as various species of *Campylobacter* (8).

*Lawsonia intracellularis* is an intracellular, possibly obligate intracellular, bacterium. It can only be cultured *in vitro* with tissue culture cells (9, 26). Pigs suffering from PPE are characterised by multiple abnormal immature crypts and *L. intracellularis* is located in the cytoplasm of these cells.

PPE is a significant cost component associated with the pig industry, especially in terms of stock losses, medication costs, reduced growth rates of pigs and increased feed costs. PPE also contributes to downstream indirect costs in, for example, additional labour costs and environmental costs in dealing with antibiotic residue contamination and in control measures to prevent the organism being passed on or carried to other animals or humans.

Current control strategies for PPE rely on the use of antibiotics. However, such a strategy is considered to be short to medium term especially as governmental regulatory pressures tend to target animal husbandry practices which are only supported by prophylactic antibiotics. There is a need, therefore, to develop effective, safe and low cost alternatives to the use of antibiotics. There is also a need to extend this alternative to antibiotics to similar organisms which infect other animals such as humans.

In work leading up to the present invention, the inventors sought to develop vaccines for the prophylaxis and treatment of PPE in animals and birds. The vaccines of the present invention provide an efficacious alternative to the use of antibiotics with a range of consequential husbandry and medical benefits.

5

Accordingly, one aspect of the present invention provides a vaccine composition for the prophylaxis and/or treatment of infection in an animal or bird by *L. intracellularis* or related microorganism, said vaccine composition comprising at least one immunogenic component from *L. intracellularis* or related microorganism and one or more carriers,  
10 diluents and/or adjuvants suitable for veterinary use.

The present invention is particularly useful and is exemplified hereinafter in relation to the protection and/or treatment of pigs from infection with *L. intracellularis*. However, this is done with the understanding that the present invention extends to the prophylaxis  
15 and treatment of all animals including humans and birds from infection with *L. intracellularis* and/or related microorganisms. Animals contemplated by the present invention include but are not limited to humans, companion animals (e.g. cats, dogs), livestock animals (e.g. pigs, sheep, cattle, horses, donkeys, goats), laboratory test animals (e.g. mice, rats, guinea pigs, rabbits) and captive wild animals (e.g. kangaroos, foxes,  
20 deer). The present invention also extends to birds such as poultry birds, game birds and caged birds.

Furthermore, the present invention extends to all isolates and sub-types of *L. intracellularis* as well as other species of the genus *Lawsonia* or other microorganisms  
25 related at the nucleotide, biochemical, structural, physiological and/or immunointeractive level. Reference hereinafter to "*Lawsonia intracellularis*" or its abbreviation "*L. intracellularis*" includes all microorganisms related to this microorganism. For example, a related organism may have a nucleotide sequence similarity at the chromosome or extrachromosomal level of at least about 60%, more preferably at least about 70% and  
30 even more preferably greater than at least about 80% with respect to all or part of a nucleotide sequence within the chromosome or extrachromosomal elements of *L. intracellularis*. For example, these percentage similarities may relate to the sequence set

forth in SEQ ID NO:7. This sequence is a portion of the *L. intracellularis* chromosome.

Accordingly, this aspect of the present invention is directed to a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis*, said vaccine  
5 composition comprising at least one immunogenic component from *L. intracellularis* and one or more carriers, diluents and/or adjuvants suitable for veterinary use.

The term "immunogenic component" refers to a component of *L. intracellularis* including a peptide, polypeptide or a protein encoded by DNA from or derived from *L.*  
10 *intracellularis* which is capable of inducing a protective immune response in a pig. A protective immune response may be at the humoral and/or cellular level and generally results in a substantial reduction in the symptoms of PPE in pigs. The vaccine compositions will comprise an effective amount of immunogenic component such as to permit induction of a protective immune response.

15 Accordingly, the present invention is directed to a vaccine composition for the prophylaxis and treatment of a pig by *L. intracellularis*, said vaccine composition comprising an amount of at least one immunogenic component from *L. intracellularis* effective to induce a protective immune response in said pig against *L. intracellularis*,  
20 said vaccine composition further comprising one or more carriers, adjuvants and/or diluents suitable for veterinary use.

The immunogenic component may be a naturally occurring peptide, polypeptide or protein, a carbohydrate, lipid or nucleic acid (e.g. DNA) or any combination thereof  
25 isolated from *L. intracellularis* or a cell culture thereof or a recombinant form of a peptide, polypeptide or protein encoded by DNA from or derived from *L. intracellularis* or is a derivative of said peptide, polypeptide or protein.

An isolated component of *L. intracellularis* is a component which has undergone at least  
30 one purification step or which has undergone at least partial concentration from a cell culture comprising *L. intracellularis* or from a lysed preparation of *L. intracellularis* cells. The purity of such a component from *L. intracellularis* which has the requisite

immunogenic properties is preferably at least about 40%, more preferably at least about 50%, even more preferably at least about 60%, still more preferably at least about 70% and even more preferably at least about 80-90% or greater relative to other components in a preparation as determined by molecular weight, immunogenic activity or other  
5 suitable means.

A particularly useful form of the vaccine is a whole cell vaccine which comprises *L. intracellularis* in attenuated form or killed cells or various fractions thereof.

10 In one preferred embodiment, the vaccine comprises killed *L. intracellularis* cells prepared, for example, by heat, formalin or other chemical treatment, electric shock or pressure and such a vaccine is particularly useful in the practice of the present invention.

According to this aspect of the present invention there is provided a vaccine composition  
15 for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis*, said vaccine composition comprising a killed preparation of *L. intracellularis* or an immunogenic fraction thereof and one or more carriers, diluents and/or adjuvants suitable for veterinary use.

20 In an alternative embodiment, a recombinant vaccine may be employed. The recombinant vaccine may comprise one or more recombinant peptides, polypeptides or proteins derived from *L. intracellularis* or is a recombinant molecule immunologically related to a peptide, polypeptide or protein derived from *L. intracellularis* or may be a fusion molecule having a first portion comprising a peptide, polypeptide or protein  
25 derived from *L. intracellularis* and a second heterologous peptide, polypeptide or protein which may be useful, for example, as a carrier molecule or an adjuvant or an immune stimulating molecule such as cytokine. A particularly useful recombinant protein from *L. intracellularis* comprises a peptide, polypeptide or protein derived from the cell surface or membrane of *L. intracellularis*, is an enzyme in a metabolic pathway within  
30 *L. intracellularis* or is a refolding and/or heatshock protein. In a preferred embodiment, the protein is a refolding/heatshock protein such as GroEL and GroES.

According to a preferred embodiment, the present invention relates to a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis*, said vaccine composition comprising at least one recombinant peptide,  
5 polypeptide or protein from *L. intracellularis* and wherein said recombinant peptide, polypeptide or protein is capable of inducing a protective immune response against *L. intracellularis* in pigs, the vaccine composition further comprising one or more carriers, diluents and/or adjuvants suitable for veterinary use.

10 In a particularly preferred embodiment, the recombinant protein is GroEL having an amino acid sequence as set forth in SEQ ID NO:2 or is a protein having an amino acid sequence with at least about 40%, at least about 60%, or more preferably at least about 70% and even more preferably at least about 80-90% or greater similarity to all or part of the amino acid sequence set forth in SEQ ID NO:2.

15

In another embodiment, the recombinant molecule is GroES having an amino acid sequence as set forth in SEQ ID NO:4 or is a molecule having an amino acid sequence at least about 40%, at least about 60%, more preferably at least about 70% and even more preferably at least about 80-90% or greater similarity to all or part of the amino  
20 acid sequence set forth in SEQ ID NO:4.

The present invention further extends to a vaccine comprising a recombinant vaccine vector encoding a peptide, polypeptide or protein derived from *L. intracellularis* as described above. The vaccine vector may be of viral, yeast or bacterial origin and  
25 would be capable of expression of a genetic sequence encoding a peptide, polypeptide or protein from *L. intracellularis* in a manner effective to induce a protective immune response. For example, a non-pathogenic bacterium could be prepared containing a recombinant sequence capable of encoding a peptide, polypeptide or protein from *L. intracellularis*. The recombinant sequence would be in the form of an expression vector  
30 under the control of a constitutive or inducible promoter. The bacterium would then be permitted to colonise suitable locations in a pig's gut and would be permitted to grow and produce the recombinant peptide, polypeptide or protein in amount sufficient to



induce a protective immune response against *L. intracellularis*.

In a further alternative embodiment, the vaccine may be a DNA vaccine comprising a DNA molecule encoding a peptide, polypeptide or protein from *L. intracellularis* and  
5 which is injected into muscular tissue or other suitable tissue in a pig under conditions sufficient to permit transient expression of said DNA to produce an amount of peptide, polypeptide or protein effective to induce a protective immune response.

The vaccines of the present invention may contain a single peptide, polypeptide or  
10 protein or a range of peptides, polypeptides or proteins covering different or similar epitopes. In addition, or alternatively, a single polypeptide may be provided with multiple epitopes. The latter type of vaccine is referred to as a polyvalent vaccine. A multiple epitope includes two or more repeating epitopes.

15 The formation of vaccines is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pennsylvania, USA.

The present invention, therefore, contemplates a pharmaceutical composition or vaccine  
20 composition comprising an immunity developing effective amount of one or more of:

- (i) an immunogenic component from *L. intracellularis*;
- (ii) a recombinant peptide, polypeptide or protein from *L. intracellularis* having immunogenic properties; and/or
- 25 (iii) whole cells or a component or fraction thereof from *L. intracellularis*.

The above components are referred to hereinafter as "active ingredients". The active ingredients of a vaccine composition as contemplated herein exhibit excellent therapeutic activity, for example, in the treatment and/or prophylaxis of PPE when administered in  
30 an amount which depends on the particular case. For example, for recombinant molecules, from about 0.5 µg to about 20 mg per kilogram of body weight per day may be administered. Dosage regime may be adjusted to provide the optimum therapeutic

response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. Booster administration may also be required.

- 5 The active ingredients may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or implanting (eg using slow release technology). Depending on the route of administration, the active ingredients which comprise, for example, peptides, polypeptides or proteins may be required to be coated in a material to protect said  
10 ingredients from the action of enzymes, acids and other natural conditions which may inactivate said ingredients.

The term "adjuvant" is used in its broadest sense and includes any immune stimulating compound such as interferon. Adjuvants contemplated herein include  
15 resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether and Freund's complete and incomplete adjuvant.

The active compounds may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures  
20 thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous  
25 preparation of sterile injectable solutions or dispersion. In all cases the form must be fluid to the extent that easy syringability exists unless the pharmaceutical form is a solid or semi-solid such as when slow release technology is employed. In any event, it must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms.

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The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

Carriers and diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents in vaccines is well known in the art. Except insofar as any conventional media or agent is incompatible with an active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

30

Still another aspect of the present invention is directed to antibodies to the peptides, polypeptides or proteins from *L. intracellularis* or recombinant forms thereof or non-proteinaceous molecules such as carbohydrates. Such antibodies may be monoclonal or  
5 polyclonal and may be selected from naturally occurring antibodies to *L. intracellularis* or may be specifically raised to specific molecules or whole cells or components or fractions thereof. The antibodies of the present invention are particularly useful for immunotherapy and vaccination and may also be used as a diagnostic tool for infection or for monitoring the progress of a vaccination or therapeutic regime.

10

For example, recombinant *L. intracellularis* peptides, polypeptides or proteins can be used to screen for naturally occurring antibodies to *L. intracellularis*. Alternatively, specific antibodies can be used to screen for *L. intracellularis*. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA.

15 Hereinafter, an immunogenic component is considered to encompass an immunogenic component of *L. intracellularis* and includes recombinant molecules, whole cells and cell extracts.

In accordance with this aspect of the present invention, the immunogenic components  
20 are particularly useful in screening for antibodies to *L. intracellularis* and, hence, provide a diagnostic protocol for detecting *L. intracellularis* infection. Alternatively, biological samples can be directly screened for *L. intracellularis* using antibodies raised to immunogenic components.

25 Accordingly, there is provided a method for the diagnosis of *L. intracellularis* infection in a pig comprising contacting a biological sample from said pig with an immunogenic component binding effective amount of an antibody for a time and under conditions sufficient for an immunogenic component-antibody complex to form, and then detecting said complex.

30

The presence of immunogenic components (or antibodies thereto) in a pig's blood, serum, or other bodily fluid, can be detected using a wide range of immunoassay techniques such as those described in US Patent Nos. 4,016,043, 4,424,279 and  
5 4,018,653. This includes both single-site and two-site, or "sandwich", assays of the non-competitive types, as well as in the traditional competitive binding assays. Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention.

10

Briefly, in a typical forward assay, an immunogenic component-specific antibody is immobilised onto a solid substrate to form a first complex and the sample to be tested for immunogenic component brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an  
15 antibody-immunogenic component secondary complex, a second immunogenic component antibody, labelled with a reporter molecule capable of producing a detectable signal, is then added and incubated, allowing sufficient time for the formation of a tertiary complex. Any unreacted material is washed away, and the presence of bound labelled antibody is determined by observation of a signal produced by the reporter  
20 molecule. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparing with a control sample. The present invention contemplates a range of variations to the subject assay including an assay for *L. intracellularis* antibodies using, for example, recombinant peptides, polypeptides or proteins from this organism.

25

The solid substrate is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known  
30 in the art and generally consist of cross-linking covalently binding or physically adsorbing the molecule to the insoluble carrier.

- By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, produces an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecule in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase,  $\beta$ -galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates, which yield a fluorescent product.
- Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining ternary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.
- The present invention is further described by the following non-limiting Figures and/or Examples.

In the Figures:

**Figure 1** is a representation of the nucleotide sequence [SEQ ID NO:1] and corresponding amino acid sequence of GroEL [SEQ ID NO:2].

5

**Figure 2** is a representation of the nucleotide sequence [SEQ ID NO:1] and corresponding amino acid sequence of GroES [SEQ ID NO:2].

**Figure 3** is a photographic representation showing Western analysis of *L. intracellularis* antigens recognised by vaccinated pigs. Track 1 was probed with pig sera from a pig (395) that had been immunised three times with the formalin killed whole *L. intracellularis* vaccine. Track 2 to 5 were probed with sera obtained from pigs Y10, Y12, Y14 and Y16, respectively on day 0.

10 **Figure 4** is a photographic representation of the small intestine obtained from pig Y1 on day 20.

**Figure 5** is a photographic representation of the small intestine obtained from pig Y2 on day 20.

20

**Figure 6** is a photographic representation of the small intestine obtained from pig Y4 on day 20.

**Figure 7** is a graphical representation showing end titres of GroEL antibodies in the serum of pigs at day 2 □ and day 17 ■ of the vaccine trial.

25

**Figure 8** is a graphical representation showing T-cell proliferation responses to GroEL and GST proteins by peripheral blood lymphocytes obtained from pigs on day 2 ■ rGroEL □ GST.

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**Figure 9** is a schematic representation of the plasmid pET-EL2 which contains the GroEL gene from *L. intracellularis*.

**Figure 10** is a schematic representation of the plasmid pCIGH-EL2 which contains the GroEL gene from *L. intracellularis* driven by the CMU promoter.

The following single and three letter abbreviations are used for amino acid residues:

5

	Amino Acid	Three-letter Abbreviation	One-letter Symbol
10	Alanine	Ala	A
	Arginine	Arg	R
	Asparagine	Asn	N
	Aspartic acid	Asp	D
	Cysteine	Cys	C
15	Glutamine	Gln	Q
	Glutamic acid	Glu	E
	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
20	Leucine	Leu	L
	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
25	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V
30	Any residue	Xaa	X



## EXAMPLE 1

### SOURCES OF PIG TISSUE

#### 5   **Infected Pig Intestines**

Sections of grossly thickened ilea were taken from pigs naturally or experimentally affected by PPE. The presence of *L. intracellularis* bacteria in the ilea was confirmed using modified acid-fast Ziehl-Neelson staining, and/or immunofluorescent staining with specific monoclonal antibodies (10). An example of a suitable antibody is monoclonal  
10   antibody IG4 available from the University of Edinburgh, UK.

#### **Normal Pig Intestines**

Ilea from six pigs not infected with the disease were obtained from a quarantined piggery which has had no recorded outbreak of proliferative enteritis (Victorian Institute  
15   of Animal Science, Werribee, Australia).

## EXAMPLE 2

### ISOLATION OF *LAWSONIA INTRACELLULARIS* BACTERIA FROM THE INFECTED PIG ILEUM

20

*Lawsonia intracellularis* bacteria were extracted directly from lesions of proliferative enteritis in pigs by filtration and further purified over a percoll (Pharmacia, Uppsala, Sweden) gradient. Infected ilea were collected from pigs and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum  
25   were thawed and approximately 8g of infected mucosa were scraped from the intestinal wall. The mucosa was homogenised with 40 ml sterile phosphate buffered saline (PBS) on half speed for 10 s using a Sorvall omnimixer. This suspension was centrifuged at 2000 xg for 4 minutes. The supernatant was discarded and the cell pellet was resuspended in 40 ml PBS and recentrifuged. This washing step was repeated twice.  
30   The cell pellet was then resuspended in 20 ml PBS and homogenised on top speed for one minute to release *L. intracellularis* bacteria.

This homogenate was centrifuged at 1000 xg for 4 minutes giving a pellet containing a crude mixture of homogenised epithelial cells and intestinal bacteria. The supernatant was filtered using filters with pore sized 3 mm, 1.2 mm and 0.8 mm (Millipore Corporation, MA, USA). The filtrate was centrifuged at 8000 xg for 30 minutes, resulting in a small pellet of *L. intracellularis* bacteria. The *L. intracellularis* bacteria were further purified using a 45% self forming percoll gradient as follows: 2 mls of the bacterial preparation was mixed by inversion into 30 mls of a 45% self forming Percoll (Pharmacia LKB, Uppsala, Sweden) gradient (45% v/v of Percoll, 150 mM NaCl). The gradients were centrifuged in a Sorval centrifuge using the SS34 rotor, at 20,000rpm for 30 minutes at 4°C. Usually a number of bands form within the gradient. The band (usually located approx. 10-20mm from the base of the tube) containing the *L. intracellularis* bacteria was collected and the volume made up to 16 mls with PBS. The solution was then centrifuged for 15 minutes at 8000rpm. The resultant pellet was washed with PBS before being resuspended in a final volume of approximately one ml.

15

### EXAMPLE 3

#### PURIFICATION OF *LAWSONIA INTRACELLULARIS* GENOMIC DNA

*Lawsonia intracellularis* genomic DNA was purified from scrapings taken from normal and infected pig ilea using a percoll gradient purification method described by Anderson *et al* (11) and Sambrook *et al* (12).

20

### EXAMPLE 4

#### IMMUNOSCREENING OF GENOMIC LIBRARIES

25

A lambda ZAP *L. intracellularis* genomic library was plated on a lawn of *Escherichia coli* BB4 (23) cells at a density of 10,000 plaque-forming units (pfu) per 150 mm L-broth agar plate. The library was screened with a rabbit or pig anti- *L. intracellularis* sera using the method described in the Protoblot Technical Manual (Promega, WI, USA). Filters were blocked in a buffer containing 10mM Tris HCl, pH8.0, 150mM NaCl, 0.05% Tween 20, 1% w/w gelatin. Positive plaques identified in a primary screen were picked, replated at a lower density and rescreened until individual positive plaques

30

were identified.

#### EXAMPLE 5

#### ISOLATION AND SEQUENCING OF cDNA INSERTS

5

Phagemid DNA from positive  $\lambda$ ZAP phage clones was isolated by excision in vivo of the pBluescript phagemid under the conditions recommended by Stratagene (CA, USA). Plasmid DNA was extracted by the method of Birnboim and Doly (21). DNA sequencing of cDNA inserts was performed by the chain termination method (22).

10

#### EXAMPLE 6

#### ANTISERA

Antisera to *L. intracellularis* bacteria were raised in rabbits and pigs. Rabbits were  
15 injected intramuscularly with a preparation of percoll gradient-purified *L. intracellularis* bacteria mixed with a double-emulsion made by processing with oil adjuvant (Freund's incomplete adjuvant, CSL Limited, Melbourne, Australia), and then with Tween 80 enhancer. Two 3 ml injections, containing 9 mg protein, were given four weeks apart. Blood samples were collected from the ear vein prior to immunisation and two weeks  
20 following the second injection.

A 6-week old pig was hyperimmunised by intramuscular injection of percoll gradient purified *L. intracellularis* bacteria prepared with Freund's incomplete adjuvant as for the rabbit. Three injections of the prepared antigen were administered four weeks apart, and  
25 blood was collected from the vena cava vein two weeks following the final injection. Serum samples were also collected from six 4-week old pigs prior to challenge with *L. intracellularis* and three weeks post challenge when pigs were shedding *L. intracellularis* bacteria in their faeces. Diluted pig sera (1 ml, 1 in 1000) were pre-absorbed with 100 ml *E. coli* DH5 $\alpha$  (24) lysate for 1 h at room temperature with gentle mixing. The lysate  
30 was prepared by freeze-thawing a suspension of *E. coli* in PBS.

### EXAMPLE 7

#### SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

5

Protein samples were resuspended in 250 ml of sample buffer (62.4 mM HCl, 2% w/v SDS, 10% v/v glycerol, 5% v/v 20 mercaptoethanol, 0.002% bromophenol blue, pH 6.8) and heated to 95°C for 5 minutes before separating solubilised proteins electrophoretically on a 0.1% w/v SDS-12% w/v PAGE vertical slab gel (13).

10

### EXAMPLE 8

#### WESTERN BLOTTING

15 Proteins were electrophoretically transferred to Immobilon-P (Millipore Corporation, MA, USA) membranes in a Trans-Blot Cell (BioRad, CA, USA) at 100 V for 1 h in a buffer containing CAPS (3-[Cyclohexylamino]-1-propanesulfonic acid, pH 11, Sigma, MI, USA) and 10% v/v methanol. The membranes were then blocked with 10% w/v Blotto (Diploma skim milk powder, Melbourne, Australia) in PBS containing 0.05% v/v Tween-20 (PBST) for 1 h at room temperature with gentle rocking. The filters were  
20 washed once in 0.1% w/v Blotto, PBST (50 ml) for 5 min, and transferred to antisera diluted in 1% w/v Blotto, PBST. Rabbit antisera was diluted 1 in 2000 and pre-absorbed pig antisera was diluted 1 in 1000. The filters were incubated in either rabbit or pig antisera for 1 h followed by washing twice as before.

25 Peroxidase-conjugated goat anti-rabbit immunoglobulins (Silenus, Melbourne, Australia), diluted 1:3000 in 1% w/v Blotto, PBST were used to detect rabbit antibodies. Similarly, HRP conjugated anti-swine immunoglobulins (DAKO, CA, USA) were applied at a dilution of 1:1000. Enhanced Chemiluminescence (ECL, Amersham, IL, USA) was used to discriminate *L. intracellularis* proteins. Prior to ECL detection, blots were washed  
30 as before for 1 h, changing the wash solution every 15 min. The filters were exposed to autoradiographic film (Agfa, NJ, USA) for less than 1 minute before developing.

## EXAMPLE 9

### PRODUCTION OF RECOMBINANT GroEL

Clones found to be positive according to the immunoscreening method described in  
5 Example 4 were sequenced using the protocol detailed in Example 5. One clone isolated  
represented the GroEL protein. The nucleotide [SEQ ID NO:1] and corresponding  
amino acid [SEQ ID NO:2] of GroEL are shown in Figure 1. Another clone isolated  
represented the GroES protein. The nucleotide sequence of GroES [SEQ ID NO:3] is  
shown in Figure 2. The corresponding amino acid sequence is defined in SEQ ID NO:4.  
10 The term "rGroEL" and "rGroES" refer to recombinant forms of GroEL and GroES,  
respectively.

The coding region of *L. intracellularis* GroEL was amplified using the following  
primers; Primer one: CGAGGATCCGATGGCTTCTAAAGAAATCC (SEQ ID NO:5)  
15 and Primer two: GGAATTCGGATCCCTAGTACATACCGTCCAT (SEQ ID NO:6).  
The resulting PCR product was digested with *Bam*HI and cloned into *Bam*HI site of  
pBS. The resulting clone was called pBS-EL2. The *Bam*HI insert of pBS-EL2 was then  
cloned into pET15b. The resulting clone which contained the GroEL transcript in the  
correct orientation was named pET-EL2. An overnight culture of *E. coli* BL21 (25)  
20 transformed with pET-EL2 was diluted 1:10 in L-broth ampicillin, 1 mM IPTG and  
grown at 37°C with shaking at 200rpm for 2 hours. The bacterial pellet was recovered  
by centrifugation and the bacteria were then lysed by a freeze thaw cycle. Recombinant  
GroEL protein was purified under denaturing conditions over a nickel charged column  
using the conditions recommended by the manufacturer (Novagen, WI, USA). Purified  
25 protein was confirmed to be GroEL by SDS-PAGE followed by Western analysis, as  
described in Examples 7 and 8 using a rabbit anti *L. intracellularis* sera. The purified  
recombinant GroEL was dialysed against PBS and then repurified by nickel charged  
column chromatography under non-denaturing conditions. A centricon 10 (Amicon, MA,  
USA) column was then used to buffer exchange into PBS and concentrate the protein.

30

### EXAMPLE 10

#### CONSTRUCTION OF pCIGH-EL2

5 The coding region of GroEL was excised from pBS-EL2 and cloned into the *Bam*HI site of the DNA vaccine vector, pCIGH. In this construct GroEL expression is directed by the CMV promoter. pCIGH containing *L. intracellularis* GroEL in the correct orientation was named pCIGH-EL2. To confirm that pCIGH-EL2 correctly encoded for the expression of *L. intracellularis* GroEL, pCIGH-EL2 was transfected into COS cells  
10 using standard techniques and expression of GroEL protein was confirmed by SDS-PAGE followed by western analysis, described in Example 8, using the rabbit anti *L. intracellularis* sera (Example 6). *Escherichia coli* carrying pCIGH-EL2 was deposited at the Australian Government Analytical Laboratories, Pymble, New South Wales, 2073, Australia on 28 November, 1995 under Accession Number N95/72592.

15

### EXAMPLE 11

#### PRODUCTION OF pCIGH-EL2 DNA FOR VACCINATION

For vaccine immunisations pCIGH-EL2 was purified in the following manner. *E. coli*  
20 DH5 $\alpha$  cells transformed with pCIGH-EL2 were grown overnight at 37°C with shaking in one litre LB-broth cultures containing ampicillin. The bacterial pellet was recovered by centrifugation at 10,000 g for 10 minutes. The plasmid DNA was then purified by alkaline lysis (21). Endotoxin was removed by triton X-114 phase extraction as described by Manthorpe et al (19).

25

### EXAMPLE 12

#### rGroEL ELISA

Recombinant GroEL at a concentration of 1-5 $\mu$ g/ml in PBS was incubated in the wells  
30 of a microtitre plate overnight at 4°C. The wells were then blocked by the addition of 5% W/V blotto for one hour at room temperature. The wells were then washed with PBST three times before serial dilutions of pig sera was added to each well. Following

a one hour incubation at room temperature the wells were washed three times with PBST before 50 ul of a 1:3000 dilution of goat anti pig immunoglobulins conjugated with horse radish peroxidase (DAKO, CA, USA) was added. Following a one hour room temperature incubation the wells were washed three times with PBST before the HRP substrate was added. The colour was allowed to developed for 30 minutes before the optical density of each well was read at 414 nm using a Titretek Multiscan MCC.

### EXAMPLE 13

#### T-CELL RESPONSES TO rGroEL

10

Peripheral blood lymphocytes were purified from 10 mls of pig blood collected in the presence of lithium heparin by the following method. The whole blood samples were centrifuged at room temperature for 10 minutes at 2000 rpm and the buffy coat cells collected. This cell mixture, approximately 2 ml, was diluted to 4 mls by the addition of PBS. The cell mixture was then overlaid on 4 mls of ficoll-paque (Pharmacia, Uppsala, Sweden) and the tubes centrifuged at room temperature for 20 minutes at 2000 rpm. The resulting band containing the lymphocytes was recovered. The lymphocytes were washed in PBS before being resuspended in RPMI (Gibco-BRL, MD, USA) media at a concentration of  $2 \times 10^6$ /ml. An amount of 100 ul of this suspension was added to each well of a microtitre plate containing 100 ul RPMI, 20% w/v FCS, 100 units/ml Penicillin, 0.1 mg/ml Steptomycin, 2mM L-Glutamine. To each test well 1 ug of rGroEL was added. The controls used were as follows: positive control Con A 5ug/ml, negative control GST from *F. hepatica* at 1 ug/well. The microtitre plate was incubated at 37°C 10% v/v CO<sub>2</sub> for 72 hours before 1 uCi of Methly-<sup>3</sup>H Thymidine (Amersham, IL, USA) (46Ci/mmol) was added to each well. Following a further 9 hour incubation the cells were harvested using a Skatron Harvester and the amount of radioactivity of each well determined by counting in a Packcard Matrix 9600 Betacounter.

#### EXAMPLE 14

##### IMMUNOFLOUORESCENT DETECTION OF *LAWSONIA INTRACELLULARIS* BACTERIA IN PIG FAECES

5

Faecal swabs of pigs were taken using a cotton tipped swab and then the sample was smeared onto a glass slide. After allowing ten minutes for air drying the smears were heat fixed by heating to 60°C for approximately 10 seconds. The slides were then rinsed in PBS. An amount of 30ul of a 1/200 dilution of a mouse ascites containing IG4  
10 monoclonal antibody (see Example 1) was added, a glass cover slip applied, and the slides were incubated at room temperature for 40 minutes. The cover slip was removed and the slides were washed (PBST for 7 minutes, three times). An amount of 30ul of a 1/40 dilution of a FITC conjugated anti-mouse antiserum (Silenus, Melbourne Australia) was added, a glass cover slip applied and the slides were incubated at room  
15 temperature for 40 minutes. The cover slip was removed and the slides were washed (PBST X3 for 7 minutes). The slides were given a final rinse in PBS. A drop of 10% v/v glycerol PBS was added and a glass cover slip applied. The fluorescent bacteria were visualised under highpower (X1200) at 340 nm using a Lietz laborlux S microscope. Twenty fields were counted and the results (see Table 1) were expressed as the average  
20 number of *L. intracellularis* bacteria per high powered field.

#### EXAMPLE 15

##### FORMALIN-KILLED *L. INTRACELLULARIS* VACCINE

25 The percoll gradient purified bacterial *L. intracellularis* pellet was resuspended in 1 ml of 1% formal saline and incubated overnight at 4°C. The percoll gradient-purified *L. intracellularis* bacteria was then mixed into a double-emulsion made by processing with oil adjuvant (Freund's incomplete adjuvant, Commonwealth Serum Laboratories, Melbourne, Australia), and then with Tween 80 enhancer.

30



## EXAMPLE 16

### VACCINATION PROTOCOL

5 Sixteen weaned pigs (Landrace crossed with Large White) were sourced from a Pig Improvement Company piggery and treated with Neo- Terramycin (0.25 g/kilo) for 5 days. Seven days later (day -40) pigs Y1-Y8, Y10, Y12, Y14 and Y16 were vaccinated as described. Pigs Y3, Y6, Y11 and Y13 were treated for abscess with long acting terramycin on day -34.

10

The sixteen pigs were divided into four groups and treated as follows:

#### *Group 1 Negative Control Group*

Four pigs (Ear Tag No. Y1-Y4) were immunised with 200 µg of pCIGH in 200 µl of  
15 BBS intramuscularly on days -40, -26 and -12.

#### *Group 2 GroEL DNA Vaccine*

Four pigs (Ear Tag No. Y5-Y8) were immunised with 200 µg of pCIGH-EL2 DNA intramuscularly on days -40, -26 and -12.

20

#### *Group 3 Whole Bacteria Vaccine*

Four pigs (Ear Tag No. Y10, Y12, Y14 and Y16) were immunised with formalin killed *L. intracellularis* bacteria emulsified in 0.5 ml of PBS/Freunds incomplete on days -33 and -12.

25

#### *Group 4 Uninfected Controls*

Four pigs (Ear Tag No. Y9, Y11, Y13 and Y15) received no treatments and were housed in a separate area from the vaccinated and orally challenged pigs.

30 The results of the vaccination are shown in Table 1.

### EXAMPLE 17

#### ORAL CHALLENGES OF INFECTED PIGS

- 5 Infected ilea were collected from pigs vaccinated as described in Example 16 and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum were thawed and approximately 150g of infected mucosa was scraped from the intestinal wall. The mucosa was homogenised with an equal volume of sterile PBS on half speed for 20 s using a Sorvall ominimixer. This suspension was diluted  
10 two fold with sterile PBS to form the challenge suspension.

On day 0 each pig from Groups 1-3 was dosed with a 5% Na Bicarb solution (10 ml/kg) followed by 30 ml of the challenge suspension. This was repeated on day 1 and day 2.

- 15 From day 11 onwards, the number of *L. intracellularis* bacteria in each pig's faeces was monitored by immunofluorescence. Pigs were monitored for signs of disease and shedding of *L. intracellularis* bacteria. Pigs shedding greater than 100 bacteria per high powered field and scouring were killed for ethical reasons.
- 20 On day 22 the surviving pigs were humanely killed and the small intestines were recovered. Two sections of small intestine were removed 5 cms and 17 cms proximally from the ileocaecal junction. These sections were fixed in 10% v/v formalin, wax embedded and sections were sent to an independent veterinary pathologist for analysis.

25

### EXAMPLE 18

#### LAWSONIA INTRACELLULARIS PROTEINS RECOGNISED BY VACCINATED PIGS

- Antibodies raised to *L. intracellularis* proteins post vaccination by pigs were analysed by Western blotting followed by ECL (Amersham, IL, USA) detection as described in  
30 Example 8. The results are shown in Figure 3. Vaccinated pigs produce antibodies to a range of *L. intracellularis* proteins. The most immunodominant proteins recognised are approximately 62.7 Kda, 58.7 Kda, 57.2 Kda, 44 Kda, 36.7 Kda and two smears from

24-26 Kda and 22-23.5 Kda. Minor immunoreactive bands had approximately the following molecular weights 67 Kda, 52.5 Kda, 50.5 Kda, 50 Kda, 48.2 KDa, 47.9 Kda, 44.7 Kda, 43.5 Kda, 42.5 Kda, 41.5 Kda, 40.5 Kda, 39 Kda, 35.3 Kda, 17 Kda, 15.5 Kda, 12 Kda and 7 Kda. The molecule weight of the proteins recognised will vary by up to 5% depending on the method used for estimation.

#### EXAMPLE 19

##### SHEDDING OF *L. INTRACELLULARIS* BACTERIA BY PIGS DURING THE TRIAL

Three of the pigs vaccinated with vector alone (Group 1 Nos. Y1, Y2 and Y4) shed greater than 100 *L. intracellularis* bacteria per high powered field in their faeces by day 19 post oral challenge (Table 1). Two of these pig (Y2 and Y4) had a bloody scour. All three pigs were humanely killed on day 20. Y3 shed low levels of *L. intracellularis* bacteria during the course of the infection trial. Maximal bacterial shedding for Y3 was 16 bacteria per high powered field.

All pigs in group 2 vaccinated intramuscularly with 200 ug of the pCIGH-EL2 DNA shed *L. intracellularis* bacteria. The amount of bacterial shedding was much reduced relative to the pigs in group 1. Maximal shedding was 10, 3, 15 and 2 *L. intracellularis* bacteria per high powered field for pigs Y5-Y8 respectively.

All pigs in groups 1-3 shed *L. intracellularis* bacteria during the course of the trial. However, pigs in group 3 (formalin -killed *L. intracellularis* vaccine) never shed more than 3 *L. intracellularis* bacteria per high powered field. Vaccination with the formalin killed *L. intracellularis* vaccine reduced total bacterial shedding of *L. intracellularis* bacteria by vaccinated pigs by 98.5% when compared with group 1 pigs.

None of the group 4 pigs (uninfected controls) shed any *L. intracellularis* bacteria during the course of the trial.

**EXAMPLE 20**  
**GROSS PATHOLOGY**

5    *Group 1        -ve control group*

- Y1    Approximately 5 cm of terminal ileum was grossly thickened. No other signs of PPE were evident macroscopically. Findings are consist with intestinal adenomatosis (See Figure 4).
- 10    Y2    The intestine was found to be grossly thickened and the serosa had the characteristic cerebriform forms (Figure 5). Over 2.5 metres of the intestine was involved. The lumen of the intestine was found to contain fresh blood and fibrinous casts were evident. Proliferative haemorrhagic enteropathy.
- Y3    No gross signs of PPE were evident.
- Y4    The intestine was found to have necrotic enteritis (Figure 6). The mucosal surface was replaced with a fibrinous pseudomembrane. Oedema of the mesentery was clearly evident. Over 2.0 meters of intestine was involved.
- 15

*Group 2        GroEL DNA vaccine*

- Y5    No gross signs of PPE.
- 20    Y6    Possible thickening of the mucosa.
- Y7    No gross signs of PPE.
- Y8    No gross signs of PPE.

*Group 3        Whole L. intracellularis cell vaccine*

- 25    Y10   No gross signs of PPE.
- Y12   No gross signs of PPE.
- Y14   No gross signs of PPE.
- Y16   No gross signs of PPE.

30   *Group 4        Uninfected controls*

- Y9    No gross signs of PPE.
- Y11   No gross signs of PPE.

Y13 No gross signs of PPE.

Y15 No gross signs of PPE.

## EXAMPLE 21

5

### HISTOPATHOLOGY REPORT

Reports are based on established histopathological descriptions in Jubb, *et al* (20).

#### *Group 1 -ve control group*

- 10 Y1 Numerous microfocal/confluent lesions of Porcine Intestinal Adenomatosis (PIA) are associated with Peyers Patches.
- Y2 Serious generalised (annular) lesions of Porcine Intestinal Adenomatosis.
- Y3 No conclusive evidence of PIA. Sparse microfocal lesions suggestive of a non-specific mild reactive (reparational) hyperplasia (rather than an adenomatosis).
- 15 Y4 Severe generalised (annular) lesions of PIA.

#### *Group 2 GroEL DNA vaccine*

- Y5 The 5cm section had marked generalised PIA associated with Peyers Patches: the remaining portions of this piece of mucosa have microfocal lesions of PIA. At
- 20 20 cm generalised microfocal PIA.
- Y6 No conclusive evidence of PIA.
- Y7 A single tiny microfocus of probable PIA is associated with Peyers Patch
- Y8 No conclusive evidence of PIA.

#### *Group 3 Whole L. intracellularis cell vaccine*

- 25 Y10 No conclusive evidence of PIA.
- Y12 No conclusive evidence of PIA.
- Y14 No conclusive evidence of PIA.
- Y16 No conclusive evidence of PIA. Possible single microfocus of PIA is associated
- 30 with Peyers Patch.

*Group 4      Uninfected controls*

- Y11    No conclusive evidence of PIA.  
Y9    No conclusive evidence of PIA.  
5    Y13    Intestine was not recovered since pig was killed due to lameness at day 15.  
Y15    Diagnosis not possible because of the poor quality of the section.

**EXAMPLE 22**

**ANTIBODY RESPONSES TO GroEL INDUCED BY VACCINATION**

10

Antibody responses to GroEL were measured according to the method described in Example 12 and results are shown in Figure 7. All pigs vaccinated with pCIGH-EL2 DNA produced detectable antibodies to the GroEL protein by day 2. By day 17 pigs Y5 -Y8 had end titres of 1:1,600. No other pigs had detectable antibody to GroEL  
15    protein, except Y1 and Y2 which had titres that were just detectable.

**EXAMPLE 23**

**T-CELL RESPONSES TO GroEL INDUCED BY VACCINATION**

- 20    T-Cell response was determined according to the method described in Example 13. T-Cell proliferation in response to both GroEL and GST protein are shown in Figure 8. Peripheral blood lymphocytes purified from pigs Y5-Y8 on day 2, proliferated in response to exposure to GroEL protein. On average this response was approximately three times that seen with the negative control antigen, GST.

25

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred  
30    to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

TABLE 1

951103.p:\oper\ejh,TADC1.prv,20

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: DARATECH PTY LTD
- (ii) TITLE OF INVENTION: THERAPEUTIC AND DIAGNOSTIC  
COMPOSITIONS - B
- (iii) NUMBER OF SEQUENCES: 7
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: DAVIES COLLISON CAVE
  - (B) STREET: 1 LITTLE COLLINS STREET
  - (C) CITY: MELBOURNE
  - (D) STATE: VICTORIA
  - (E) COUNTRY: AUSTRALIA
  - (F) ZIP: 3000
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: AU PROVISIONAL
  - (B) FILING DATE: 30-NOV-1995
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: HUGHES DR, E JOHN L
  - (C) REFERENCE/DOCKET NUMBER: EJH/EK
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: +61 3 9254 2777
  - (B) TELEFAX: +61 3 9254 2770

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 759 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..759

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TCA CGA GGT GTA GAT AAA CTT GCA AAT GCT GTT AAA GTA ACA CTT GGA	96
Ser Arg Gly Val Asp Lys Leu Ala Asn Ala Val Lys Val Thr Leu Gly	
20 25 30	
CCT AAA GGC CGT AAT GTC GTT ATT GAA AAG TCT TTT GGT TCC CCA GTT	144
Pro Lys Gly Arg Asn Val Val Ile Glu Lys Ser Phe Gly Ser Pro Val	
35 40 45	
ATT ACA AAA GAT GGT GTA TCT GTT GCA AAA GAA ATT GAA CTT GAA GAT	192
Ile Thr Lys Asp Gly Val Ser Val Ala Lys Glu Ile Glu Leu Glu Asp	
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Lys Phe Glu Asn Met Gly Ala Gln Met Val Lys Glu Val Ala Pro Lys	
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ACT AGC GAT ATT GCT GGT GAT GGA ACT ACA ACA GCA ACA GTC CTT GCA	288
Thr Ser Asp Ile Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala	
85 90 95	
CAA GCT ATT TAT CGT GAA GGT GTA AAA CTT GTA GCA GCT GGT CGT AAT	336
Gln Ala Ile Tyr Arg Glu Gly Val Lys Leu Val Ala Ala Gly Arg Asn	
100 105 110	
CCT ATG GCC ATT AAA CGT GGC ATA GAT AAA GCT GTT GTT GCT GTT ACT	384
Pro Met Ala Ile Lys Arg Gly Ile Asp Lys Ala Val Val Ala Val Thr	
115 120 125	
AAA GAA CTA AGC GAC ATT ACA AAG CCT ACT CGT GAC CAA AAA GAA ATA	432
Lys Glu Leu Ser Asp Ile Thr Lys Pro Thr Arg Asp Gln Lys Glu Ile	
130 135 140	

GCT CAA GTT GGA ACC ATT TCT GCA AAC TCT GAT ACA ACA ATA GGT AAT	480
Ala Gln Val Gly Thr Ile Ser Ala Asn Ser Asp Thr Thr Ile Gly Asn	
145 150 155 160	
ATC ATA GCT GAA GCT ATG GCT AAA GTT GGA AAA GGA GGT GTT ATC ACA	528
Ile Ile Ala Glu Ala Met Ala Lys Val Gly Lys Gly Gly Val Ile Thr	
165 170 175	
GTT GAG GAA GCT AAA GGT CTT GAA ACT ACA TTA GAT GTG GTT GAA GGA	576
Val Glu Glu Ala Lys Gly Leu Glu Thr Thr Leu Asp Val Val Glu Gly	
180 185 190	
ATG AAG TTT GAC CGT GGC TAC CTC TCT CCA TAC TTT GTA ACT AAT CCT	624
Met Lys Phe Asp Arg Gly Tyr Leu Ser Pro Tyr Phe Val Thr Asn Pro	
195 200 205	
GAG AAA ATG GTT TGT GAA CTT GAT AAC CCT TAT ATC CTT TGT AAT GAG	672
Glu Lys Met Val Cys Glu Leu Asp Asn Pro Tyr Ile Leu Cys Asn Glu	
210 215 220	
AAA AAG ATT ACT AGC ATG AAA GAC ATG CTA CCA ATC TTA GAA CAA GTT	720
Lys Lys Ile Thr Ser Met Lys Asp Met Leu Pro Ile Leu Glu Gln Val	
225 230 235 240	
GCT AAA GTA AAC CGT CCA CTC CTT ATT ATT GCT GAA GAC	759
Ala Lys Val Asn Arg Pro Leu Leu Ile Ile Ala Glu Asp	
245 250	

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 253 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Ser	Arg	Gly	Val	Asp	Lys	Leu	Ala	Asn	Ala	Val	Lys	Val	Thr	Leu	Gly
			20					25					30		
Pro	Lys	Gly	Arg	Asn	Val	Val	Ile	Glu	Lys	Ser	Phe	Gly	Ser	Pro	Val
		35					40					45			
Ile	Thr	Lys	Asp	Gly	Val	Ser	Val	Ala	Lys	Glu	Ile	Glu	Leu	Glu	Asp
	50					55					60				
Lys	Phe	Glu	Asn	Met	Gly	Ala	Gln	Met	Val	Lys	Glu	Val	Ala	Pro	Lys
	65				70					75					80

Thr Ser Asp Ile Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala  
85 90 95

Gln Ala Ile Tyr Arg Glu Gly Val Lys Leu Val Ala Ala Gly Arg Asn  
100 105 110

Pro Met Ala Ile Lys Arg Gly Ile Asp Lys Ala Val Val Ala Val Thr  
115 120 125

Lys Glu Leu Ser Asp Ile Thr Lys Pro Thr Arg Asp Gln Lys Glu Ile  
130 135 140

Ala Gln Val Gly Thr Ile Ser Ala Asn Ser Asp Thr Thr Ile Gly Asn  
145 150 155 160

Ile Ile Ala Glu Ala Met Ala Lys Val Gly Lys Gly Gly Val Ile Thr  
165 170 175

Val Glu Glu Ala Lys Gly Leu Glu Thr Thr Leu Asp Val Val Glu Gly  
180 185 190

Met Lys Phe Asp Arg Gly Tyr Leu Ser Pro Tyr Phe Val Thr Asn Pro  
195 200 205

Glu Lys Met Val Cys Glu Leu Asp Asn Pro Tyr Ile Leu Cys Asn Glu  
210 215 220

Lys Lys Ile Thr Ser Met Lys Asp Met Leu Pro Ile Leu Glu Gln Val  
225 230 235 240

Ala Lys Val Asn Arg Pro Leu Leu Ile Ile Ala Glu Asp  
245 250

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 306 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..303

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG AAC CTG AAA CCT TTG AAT GAC CGT GTT TTA GTA AAA CGT CTT GAA	48
Met Asn Leu Lys Pro Leu Asn Asp Arg Val Leu Val Lys Arg Leu Glu	
1 5 10 15	
TCT GAA GAA AAA ACA GCT GGT GGA CTC TAT ATC CCT GAT ACT GCT AAA	96
Ser Glu Glu Lys Thr Ala Gly Gly Leu Tyr Ile Pro Asp Thr Ala Lys	
20 25 30	
GAA AAA CCA TCT CGT GGT GAA GTT GTT GCT GTT GGA CCT GGT AAA CAT	144
Glu Lys Pro Ser Arg Gly Glu Val Val Ala Val Gly Pro Gly Lys His	
35 40 45	
ACA GAT GAT GGT AAA TTA ATA CCT ATG GCT GTA AAA GCA GGA GAT ACA	192
Thr Asp Asp Gly Lys Leu Ile Pro Met Ala Val Lys Ala Gly Asp Thr	
50 55 60	
GTT CTT TTT AAT AAG TAT GCA GGA ACA GAA GTA AAG CTT GAT GGT GTA	240
Val Leu Phe Asn Lys Tyr Ala Gly Thr Glu Val Lys Leu Asp Gly Val	
65 70 75 80	
GAG CAT CTA GTT ATG CGT GAA GAT GAC ATC CTA GCT GTT ATT ACT GGA	288
Glu His Leu Val Met Arg Glu Asp Asp Ile Leu Ala Val Ile Thr Gly	
85 90 95	
GAA ACT GGC CGC AAG TGA	297
Glu Thr Gly Arg Lys *	
100	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 101 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

[illegible]

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGAGGATCCG ATGGCTTCTA AAGAAATCC



(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAATTCGGA TCCCTAGTAC ATACCGTCCA T

31

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 4972 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

1  AACTCCTGGT CTATCAAGAT CAACTAAAAA ATATTCTTTA TCTAATAGTT
51  GCTCAAAAAT AATTGTACCT ACAGGTAAAT GAAGAATCAA ATCTTCCCCT
101 TTTTACCAT GACGCTGGCT CCCTTTACCA CTTTCTCCAT TTTGAGCTCT
151 ATAGTGACGT TGCACACGAA AATCATAAAG GGTTAACAAA CGTGAATCAG
201 CTTTAAAAAT TATATTACCT CCATCTCCTC CATCCCCTCC ATTAGGTCCA
251 CCTTTAGGTA TAAACTTTTC GCGTCTAAAT GAAACACATC CATTTCCACC
301 TTTTCCTGCG CTCACGCTAA TAGTTACTTC ATCAACAAAA CGCATGATTA
351 TCCTTTCAAT AACAAATATC TATTCAATAC TGTTACTAAC TTGTTTACTG
401 TTTTTTCTAG AAAATTACCT GGCTAATTAT TATAGTTATA TCTAGATTAA
451 TGAAAAAGGA AGAAGTCATT ACACTCCTTC CTTATTAATA GAATCCTGGA
501 ATAATTATTA TACGGTGGGT TGTATATGCA CTCTACTATA TCTTTTACAT
551 TTACGAAAAA ATGTTTCATA AGTTACTATA CCATTAACCTT TTGCAAATAA
601 AGTATAGTCT CTTCCCATTC CAACATTTTC TCCAGGATGA ATTTTGTGAC
651 CTAGTTGACG AACAAGGATA TTGCCTGCCA AGACTTTCTG GCCGCCGAAA
701 CGCTTTATAC CACGACGTTG TCCTGGACTA TCTCTACCAT TGCGAGAACT
751 TCCACCAGCT TTCTTATGGG CCATTTTAAT ATCTCCTTAA AGCTGAATAC
801 CTGTTACTTT TAGAGCTGTA TAGTCTTGAC GATGACCTTG GAGTTTACGT
851 GAGTCATTTT TTCTCCACTT TTTAAAAACA AGAATTTTTT TATCACGACC
901 ATGCTCAAGA ACTTTAGCTA TAACTTTAGC ATTATTAATA TAGGTTGTTT
951 CAATTTGAGG AGATGAACCA CCAATCATAA AAATTTTATC AAAAAAAATT
1001 TCTGTTCCAA CTTCAGCGTC TATTTTAGAA ACAAAAATTT TAGAACCCCTC
1051 TTCAACACAG AATTGTTTTT CACCAGCTTC AATAATTGCG TACATAAATA
1101 ATGTGCCTCC CAAAAAGAC AAGAAATACT AATTTGATAT TTTCAATATT
1151 GTCAAGTAGG AACTTTATCT TTAGAATGTT AGATGTAACA ATTTTTTTAG
1201 AAAAAAATA TTTTCAATAC AATAGGAAAA GAGGAAAAAA AAAAAGATTT

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1351 TTTTATAAAA AAATTTGCAT TCCCCTCTTC CCAATTCCCA TAGAGAAGAT  
1401 TATTTATCCT AACGATTGGT GGACGCTAAG TCCCTGCTGT TTTGATTATA  
1451 TATCAAATGT TGAAACAAAT TTTGTTTAGT TTCTTTTTGT ACTCTAAAAA  
1501 GAAGACAAAA AATTCTTTAT AAAGTGTACA CTCTAAACAA AATAGTTCAC  
1551 AATAAACAGC AATACATTAT AATTAATTGG AGGATACTAT TGTCATGAAC  
1601 CTGAAACCTT TGAATGACCG TGTTTTAGTA AAACGCTCTG AATCTGAAGA  
1651 AAAACAGCT GGTGGACTCT ATATCCCTGA TACTGCTAAA GAAAAACCAT  
1701 CTCGTGGTGA AGTTGTTGCT GTTGGACCTG GTAAACATAC AGATGATGGT  
1751 AAATTAATAC CTATGGCTGT AAAAGCAGGA GATACAGTTC TTTTAAATAA  
1801 GTATGCAGGA ACAGAAGTAA AGCTTGATGG TGTAGAGCAT CTAGTTATGC  
1851 GTGAAGATGA CATCCTAGCT GTTATTACTG GAGAAACTGG CCGCAAGTGA  
1901 AAAAGGCGTA AATAAAAAGA TCGGTGATCT TTAATAATTT TATTCAGTTA  
1951 TAATGAAAAC ACTAATTACA CGCACTCTCT GAGAATTTTC TCAGAAAACT  
2001 ATATTTAACA ATTCTAAAAT CGATATGTTT TTAGGAGGAA AACCTAATG  
2051 GCTTCTAAAG AAATCCTTT TGATGCTAAA GCCCGTGAAA AACTTTCACG  
2101 AGGTGTAGAT AAAGCTGCAA ATGCTGTAA AGTAACACTT GGACCTAAG  
2151 GCCGTAATGT CGTTATTGAA AAGTCTTTTG GTTCCCCAGT TATTACAAAA  
2201 GATGGTGTAT CTGTTGCAA AGAAATTGAA CTTGAAGATA AGTTTGAAAA  
2251 TATGGGCGCT CAAATGGTTA AAGAAGTAGC TCCCAAACT AGCGATATTG  
2301 CTGGTGATGG AACTACAACA GCAACAGTCC TTGCACAAGC TATTTATCGT  
2351 GAAGGTGTAA AACTTGTAGC AGCTGGTCGT AATCCTATGG CCATTAAACG  
2401 TGGCATAGAT AAAGCTGTTG TTGCTGTTAC TAAAGAACTA AGCGACATTA  
2451 CAAAGCCTAC TCGTGACCAA AAAGAAATAG CTCAAGTTGG AACCATTCT  
2501 GCAAACCTCTG ATACAACAAT AGGTAAATATC ATAGCTGAAG CTATGGCTAA  
2551 AGTTGGAAAA GGAGGTGTTA TCACAGTTGA GGAAGCTAAA GGTCTTGAAA  
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2651 CCATACTTTG TAACATAATCC TGAGAAAATG GTTTGTGAAC TTGATAACCC  
2701 TTATATCCTT TGTAATGAGA AAAAGATTAC TAGCATGAAA GACATGTAC  
2751 CAATCTTAGA ACAAGTTGCT AAAGTAAACC GTCCACTCCT TATTATTGCT  
2801 GAAGACGTAG AAGGTGAAGC ACTTGCAACA CTTGTAGTCA ATAAGCTCCG  
2851 TGGAGCACTC CAAGTTGTAG CCGTAAAAGC TCCTGGTTTT GGTGAACGCC  
2901 GTAAAGCTAT GCTTGAAGAT ATTGCTATCC TTACTGGAGG AGAAGCAATA  
2951 TTTGAAGATC GTGGTATAAA GCTTGAAAAT GTAAGCTTGT CTTCTTTAGG  
3001 AACAGCTAAA CGTGTAGTTA TTGACAAAGA AAATACTACT ATCGTTGATG  
3051 GTGCTGAAA ATCAGAAGAT ATTAAAGCTC GAGTTAAACA AATTCGTGCA  
3101 CAAATTGAAG AAACAAGCTC AGATTATGAT CGTGAAAAAC TTCAAGAACG  
3151 TCTTGCAAAA CTTGTTGGTG GAGTAGCTGT TATCCATGTT GGAGCTGCTA  
3201 CTGAAACTGA AATGAAAGAG AAGAAGGATC GTGTAGAAGA TGCTCTAAAT  
3251 GCAACAAGAG CTGCGGTTGA AGAAGGTATT GTCCCTGGTG GTGGTACTGC  
3301 TTTTGTCCGC TCCATTAAAG TCCTTGATGA TATTAAACCT GCTGATGATG  
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3451 AGTTCGTGAA CCAAAGATG GTTTTGATT TAATGCTGCA TCAGGAGAAT  
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4201 TGCAATTACA CTAGCTGCTG AAAGAAAAAA TTGGTTAGGT ATTGCTACTG  
4251 ATATCTCTAG TGAAGCATT AAAAAAGCTA AACTTAATAG TTTAAAAAAT  
4301 AACACTCATA GTCAACTACA GTTCTTCAA TCAGATTTTA CACAACCACT  
4351 CTGTCTACCC TCTTCATTAG ACTTATATAT CAGTAATCCT CCATATATAA  
4401 GTGAAAATGA ACTGACCTCT CTTCCGCATG AAGTAATATC TTTTGAACCT  
4451 AAAATAGCTC TTACACCACA TAAATGTATT CATCTTGATG AAATAAATAC  
4501 CGTTTTACAC TGCTATAAAA AAATTATTAC CCAAGCAGAG ATATCCCTTA  
4551 AGCCTGGAGG AATAATAATT TTAGAACATG GAGCAACACA AGCAGAAGCT  
4601 ATCTTATTGT TGTTAAAAAA CAACATATGG ACAAATGTAA TAAGTCATAC  
4651 TGATCTTACA AATAAAAAATC GTTTTATTAC AGCATATAAG TATAAAATAT  
4701 AACTTAATTA TGTTGkagAa AAAACAAAAA ATAAAAATAA GATATtAAaT  
4751 ATTTtttttA aTAAAAATTAA GCAAtTACTA ATATCTTTTT TTGGrTCGtt  
4801 yaTtGsATwA GAACTTTTGG rGGrTrrCTa TGAACAAACA ACCATnCAAC  
4851 GGCCAAnTAC ATnnCAGGnT TGGGGTCATA GGGGCCACGC TTTATGTACG  
4901 TACAACCCcn ACTGAAATTC TGgnTTGnTT TGGGGGGnAA nTGGGTATCG  
4951 CAACnCTnTC CCCCCCCCCT GG

DATED this 30th day of November, 1995

DARATECH PTY. LTD. and Pig Research and Development Corporation

By Its Patent Attorneys

DAVIES COLLISON CAVE



FIGURE 1

ATG GCT TCT AAA GAA ATC CTT TTT GAT GCT AAA GCC CGT GAA AAA CTT Met Ala Ser Lys Glu Ile Leu Phe Asp Ala Lys Ala Arg Glu Lys Leu 1 5 10 15	48
TCA CGA GGT GTA GAT AAA CTT GCA AAT GCT GTT AAA GTA ACA CTT GGA Ser Arg Gly Val Asp Lys Leu Ala Asn Ala Val Lys Val Thr Leu Gly 20 25 30	96
CCT AAA GGC CGT AAT GTC GTT ATT GAA AAG TCT TTT GGT TCC CCA GTT Pro Lys Gly Arg Asn Val Val Ile Glu Lys Ser Phe Gly Ser Pro Val 35 40 45	144
ATT ACA AAA GAT GGT GTA TCT GTT GCA AAA GAA ATT GAA CTT GAA GAT Ile Thr Lys Asp Gly Val Ser Val Ala Lys Glu Ile Glu Leu Glu Asp 50 55 60	192
AAG TTT GAA AAT ATG GGC GCT CAA ATG GTT AAA GAA GTA GCT CCC AAA Lys Phe Glu Asn Met Gly Ala Gln Met Val Lys Glu Val Ala Pro Lys 65 70 75 80	240
ACT AGC GAT ATT GCT GGT GAT GGA ACT ACA ACA GCA ACA GTC CTT GCA Thr Ser Asp Ile Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala 85 90 95	288
CAA GCT ATT TAT CGT GAA GGT GTA AAA CTT GTA GCA GCT GGT CGT AAT Gln Ala Ile Tyr Arg Glu Gly Val Lys Leu Val Ala Ala Gly Arg Asn 100 105 110	336
CCT ATG GCC ATT AAA CGT GGC ATA GAT AAA GCT GTT GTT GCT GTT ACT Pro Met Ala Ile Lys Arg Gly Ile Asp Lys Ala Val Val Ala Val Thr 115 120 125	384
AAA GAA CTA AGC GAC ATT ACA AAG CCT ACT CGT GAC CAA AAA GAA ATA Lys Glu Leu Ser Asp Ile Thr Lys Pro Thr Arg Asp Gln Lys Glu Ile 130 135 140	432
GCT CAA GTT GGA ACC ATT TCT GCA AAC TCT GAT ACA ACA ATA GGT AAT Ala Gln Val Gly Thr Ile Ser Ala Asn Ser Asp Thr Thr Ile Gly Asn 145 150 155 160	480
ATC ATA GCT GAA GCT ATG GCT AAA GTT GGA AAA GGA GGT GTT ATC ACA Ile Ile Ala Glu Ala Met Ala Lys Val Gly Lys Gly Gly Val Ile Thr 165 170 175	528
GTT GAG GAA GCT AAA GGT CTT GAA ACT ACA TTA GAT GTG GTT GAA GGA Val Glu Glu Ala Lys Gly Leu Glu Thr Thr Leu Asp Val Val Glu Gly 180 185 190	576
ATG AAG TTT GAC CGT GGC TAC CTC TCT CCA TAC TTT GTA ACT AAT CCT Met Lys Phe Asp Arg Gly Tyr Leu Ser Pro Tyr Phe Val Thr Asn Pro 195 200 205	624
GAG AAA ATG GTT TGT GAA CTT GAT AAC CCT TAT ATC CTT TGT AAT GAG Glu Lys Met Val Cys Glu Leu Asp Asn Pro Tyr Ile Leu Cys Asn Glu 210 215 220	672
AAA AAG ATT ACT AGC ATG AAA GAC ATG CTA CCA ATC TTA GAA CAA GTT Lys Lys Ile Thr Ser Met Lys Asp Met Leu Pro Ile Leu Glu Gln Val 225 230 235 240	720
GCT AAA GTA AAC CGT CCA CTC CTT ATT ATT GCT GAA GAC Ala Lys Val Asn Arg Pro Leu Leu Ile Ile Ala Glu Asp 245 250	759

FIGURE 2

ISI GroES

M N L K P L N D R V L  
 A T G A A C C T G A A A C C T T T G A A T G A C C G T G T T T T A  
 V K R L E S E E K T A  
 G T A A A A C G T C T T G A A T C T G A A G A A A A A C A G C T  
 G G L Y I P D T A K E  
 G G T G G A C T C T A T A T C C C T G A T A C T G C T A A A G A A  
 K P S R G E V V A V G  
 A A A C C A T C T C G T G G T G A A G T T G T T G C T G T T G G A  
 P G K H T D D G K L I  
 C C T G G T A A A C A T A C A G A T G A T G G T A A A T T A A T A  
 P M A V K A G D T V L  
 C C T A T G G C T G T A A A A G C A G G A G A T A C A G T T C T T  
 F N K Y A G T E V K L  
 T T T A A T A A G T A T G C A G G A A C A G A A G T A A A G C T T  
 D G V E H L V M R E D  
 G A T G G T G T A G A G C A T C T A G T T A T G C G T G A A G A T  
 D I L A V I T G E T G  
 G A C A T C C T A G C T G T T A T T A C T G G A G A A A C T G G C  
 R K

CGC AAG TGA

FIGURE 3

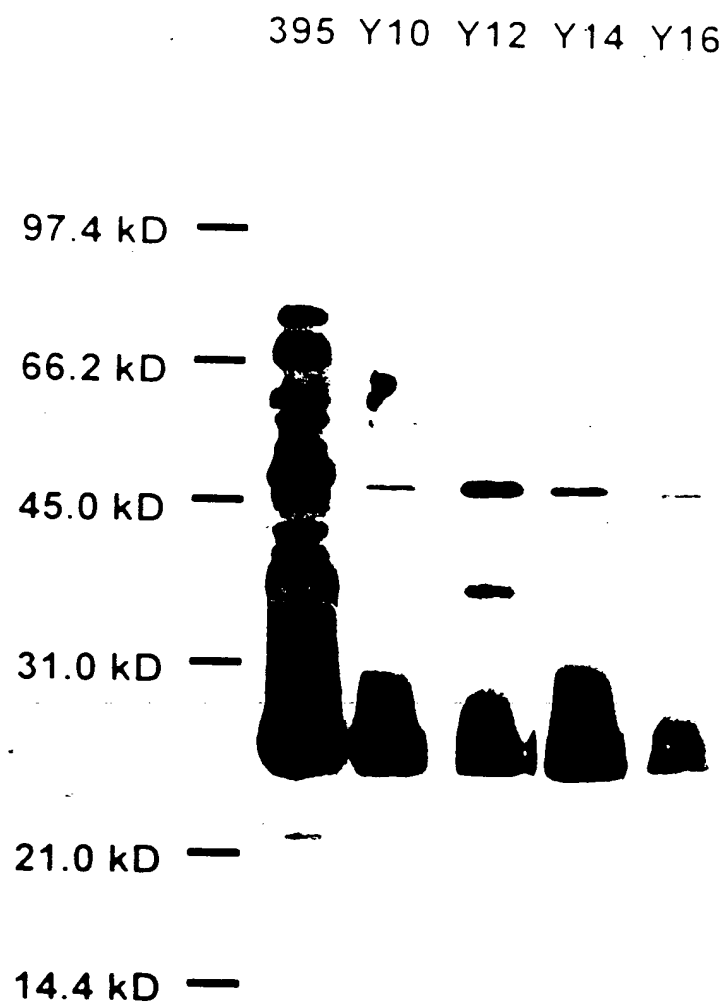


FIGURE 4



FIGURE 5

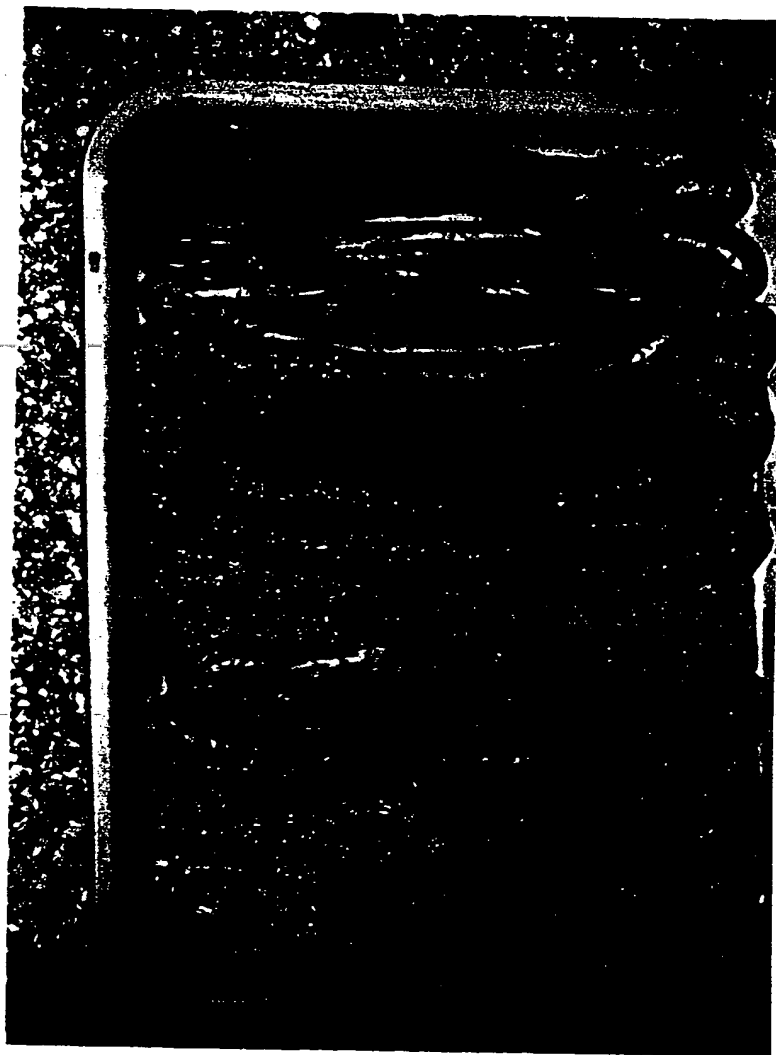
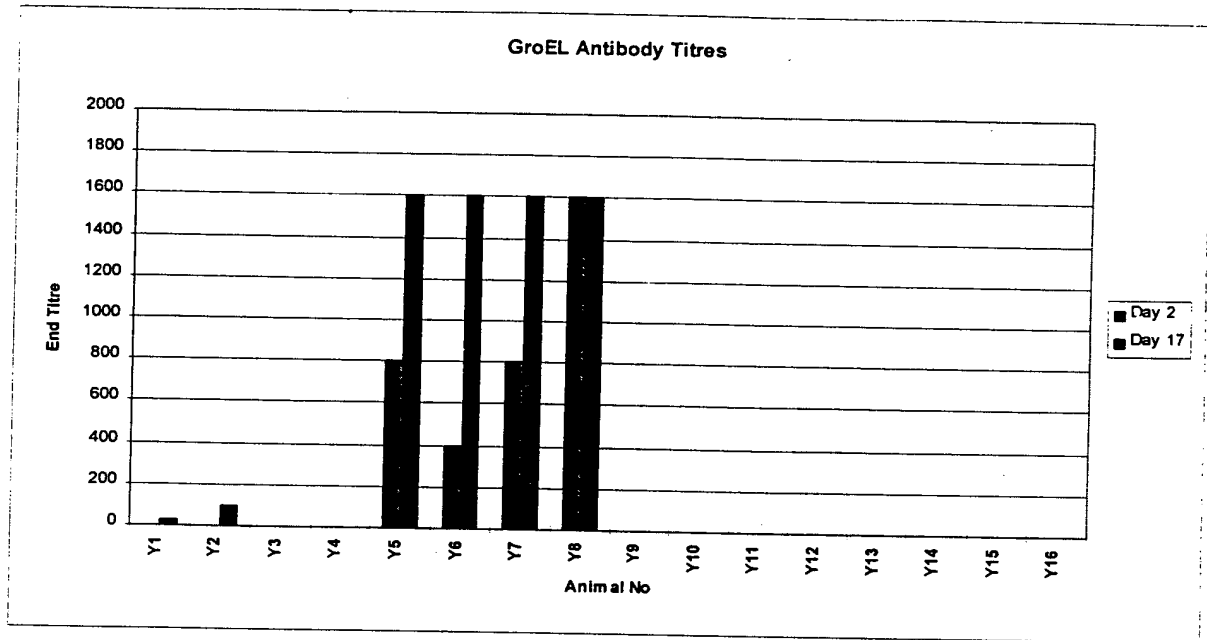




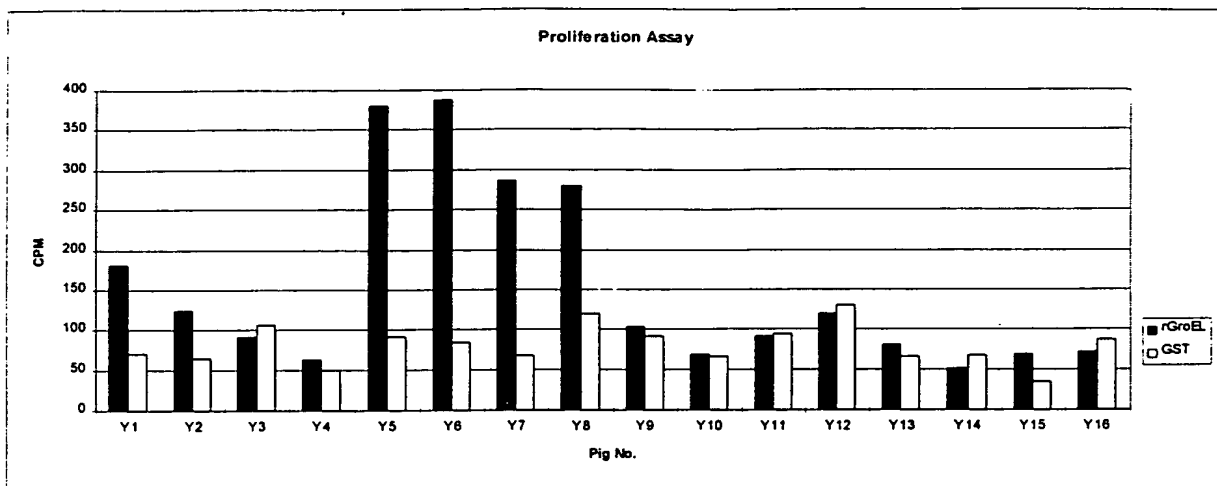
FIGURE 6





End titres of GroEL antibodies at day 2 and day 17

FIGURE 7



T-cell proliferation responses to GroEL and GST proteins by peripheral blood lymphocytes obtained from pigs on day 2.

FIGURE 8

Figure 9

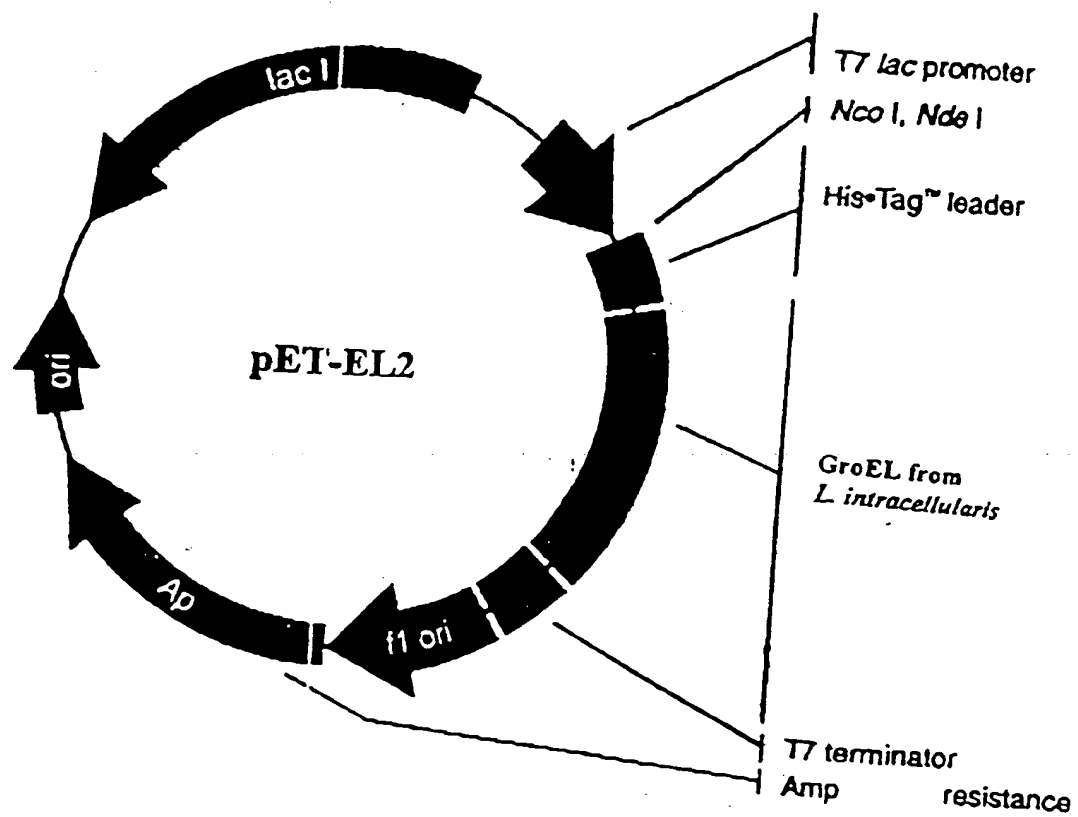


Figure 10

